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A Melanotic Tumor in the Silverside, *Menidia beryllina peninsulae* (Good and Bean).

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(Plate I; Text-fig. 1).

Introduction.

Melanotic tumors in fishes living in a feral state have been reported by several investigators. Osburn (1925) described a tumor of this type from several common bullheads (*Ameiurus nebulosus*) taken from a pond near Falmouth, Massachusetts. This investigator showed that the melanotic tumor was induced by a black pigment-producing coccus. The bacteria was isolated, cultured and the disease reproduced in the skin of apparently normal catfish by injection of the cultivated microörganisms. Other parasites are capable of causing a proliferation of melanophores. Thus, Hsiao (1941) reported a condition of melanosis in a cod (*Gadus collaris*) which was induced by the metacercariae of a heterophyid fluke. Hunter (1941) made further studies on melanophores associated with cysts of similar flukes (*Cryptocotyle lingua*) in the skin of the cunner (*Tautogolabrus adspersus*).

That melanophore proliferation may be correlated with wound healing is indicated by the experiments of Smith (1931, 1932a, 1932b) on goldfish (*Carassius auratus*). He pointed out that mechanical injuries to the skin and exposure to X-rays may cause a response of melanophores which in some cases may be so great as to cause a general but temporary melanosis.

There are other records of melanotic tumors of fishes in the literature, but the etiology of the majority of them is not known. Thus, Ingleby (1929) reported a melanotic neoplasm in an angler (*Lophius piscatorius*). It consisted of chromatophores which were of the same type as those found under the normal epithelium. The pigment cells showed a tendency to spread laterally and to deeper areas of the skin. From this description, it is apparently similar to the melanoma of platyfish-swordtail hybrids, and as will be seen shortly, to that of the silverside as well. Similar cutaneous tumors were reported by Haddow and Blake (1933) in the thornback ray.

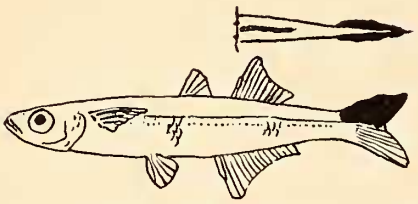
In 1931, Mr. Stewart Springer caught in Biloxi Bay at Iberville, Mississippi, a representative collection of fishes containing many individuals and species. The collection was sent to Dr. Carl L. Hubbs, Curator of Fishes in the Museum of Zoology at the University of Michigan. In sorting and cataloguing the specimens, Dr. Hubbs discovered a silverside with an apparent melanotic tumor. He kindly sent it to us for detailed study. We are indebted to him for the opportunity of making a comparative study of this tumor, which represents a spontaneous growth in a feral fish, with the melanomas of the aquarium-reared platyfish-swordtail hybrids. As Gordon (1941) has pointed out, the melanotic tumors of the platyfish-swordtail hybrids are produced experimentally by genetic methods, specifically by mating a platyfish carrying macromelanophores with a swordtail. Under natural condition in their native habitat, in the rivers of Mexico and Guatemala, *Platypoecilus maculatus* may occasionally be found living side by side with *Xiphophorus hellerii*—yet in more than 10,000 specimens examined, no hybrids and no tumors were found. Regardless of the mode of origin, it will be shown that the silverside and platyfish-swordtail hybrid melanotic tumors have many characteristics in common.

Description of the Tumor.

The silverside measured 47 mm. in total length after its preservation in alcohol. The tumor growths covered an area of $5 \times 1.5 \times 1.0$ mm. (Text-fig. 1) and they extended along the sides of the body in the dorsal region of the caudal peduncle and upper lobe of the tail fin. An examination of all external and internal parts of the body revealed no free or encysted protozoan or metazoan parasites, nor were they discovered after the tumor was sectioned. Except

for the melanotic tumor of the tail region, the fish appeared normal.

In examination of the pigmentary system of the silverside, it was noted that in the region of the lateral line a broad band of melanophores appeared to form a syncytium. Immediately above this line, large melanophores formed a striking reticular pattern merging into an even distribution of melanophores near and at the mid-dorsal line. The melanotic neoplasm was confined chiefly to the lateral line area, extending only slightly below this region. Whether or not the tumor cells were derived from the lateral line melanophores could not be determined.



TEXT-FIG. 1. Lateral and dorsal view of the tide-water silverside showing position and extent of melanoma. Slightly less than natural size. (Drawing by C. Clark.)

The tail region of the fish bearing the tumor was severed from the body and prepared for microscopical examination. After embedding in paraffin, sections were cut at 6 microns. They were stained by a variety of methods, both for bacteriological and general histological detail.

A modified method of Ziehl-Neelson for the detection of acid-fast organisms and the Brown and Brenn technique for the Gram's reaction, as outlined by Gradwohl (1938), were used. Our studies showed that the tissues appeared to be free of bacteria except for an isolated Gram negative bacillus here and there which was not regarded as significant.

Some sections were stained with Ehrlich's hematoxylin, others with Mallory's triple stain. These proved to be of greatest value although Giemsa's stain was employed as well. Histologically, the pigment cell hyperplasia in *Menidia* appeared quite like the melanotic tumors reported for the platyfish-swordtail hybrids by Reed and Gordon (1931) and indicated by them as being in the second state of melanosis. It also resembled the tumor described by Gordon and Smith (1938) in other platyfish species hybrids. The silverside tumor also has many of the features mentioned by Ingleby (1929) for the melanoma in *Lophius*.

The tumor of the silverside contained several sizes of melanin-bearing cells, corresponding somewhat to the size variation found among the melanophores in the corium in normal regions of the fish's body. The pigmented cells in the tumor mass differed from those in the normal corium in that they were not of the precise stellate form of the corial melanophores; rather they appeared amoeboid in shape.

The corium of the tumor masses was almost completely replaced by proliferating pigment-bearing cells and was considerably thickened (Pl. I, Fig. 2; B) as a result of their growth. The boundaries of the melanin-containing cells were not seen clearly owing to the presence of dense deposits of pigment granules. From our study, these apparently active cells seemed to spread laterally in the corial layer and thus might have given rise to other loci with the result that a number of pigmented nodules appeared (Pl. I, Fig. 1; 1, 2, 3). In response to the hypertrophied corial tissues, the epidermis in several places was penetrated by the tumor cells and destroyed (Pl. I, Fig. 2; A). This left the tumor naked at the surface. There was no indication of hemorrhages as described by Reed and Gordon in the platyfish-swordtail melanoma. Surrounding the region of the break-through, the epithelium of the silverside tumor was somewhat thickened and keratinized (Plate I, Fig. 2; C). The epidermal tissue contained many cells full of large clusters of melanin granules, which had some resemblance to engorged macrophages as illustrated and described by Grand, Gordon and Cameron (1941) in their study of fish melanomas in tissue cultures. The presence of large, pigment-filled cells in the epidermis appeared similar to the condition described by Smith (1932a) in the goldfish following injury and healing, and interpreted by him as being part of the process of melanin elimination by macrophages. Another instance of this phenomenon was reported by Gordon and Lansing (1943) in platyfish hybrids.

The silverside melanoma cells arising from the corial nodule penetrated (Pl. I, Fig. 2; D), in several points, the underlying fascia and invaded the subcutaneous areas attacking muscle and bone tissues. In the invasion process the muscle fibers were split and eventually broke down, as indicated by the loss of striation and hyalinization (Pl. I, Fig. 2; E). The invasion route of the tumor cells appeared to follow the path established by the intermuscular tissues. In localized areas, where bone was reached by the tumor cells, the periosteum was also attacked, destroyed and replaced Pl. I, Fig. 2; F).

In our study of sections stained with Mallory's, sinusoids and capillaries were evident; they were abundant in regions of par-

ticularly large tumor masses. No inflammatory cells were recognizable and a similar condition was reported by Gordon and Smith (1938a) for platyfish-swordtail melanomas. In the melanotic tumor of *Lophius*, however, Ingleby reported the presence of inflammatory cells in the tissues underlying the tumor, but separated from the tumor proper by a broad layer of connective tissue.

Summary and Conclusions.

A melanoma in the caudal peduncle and tail region of a feral silverside (*Menidia*) is reported, described and compared with melanotic neoplasms of *Lophius* and platyfish-swordtail hybrids. In several important respects they are quite similar.

The lesions are characterized by an overgrowth of melanin-containing cells which are capable of infiltrating, destroying and replacing epithelium, corium, fascia, muscle and periosteum.

The etiology of the tumor is not known but parasitological and bacteriological techniques employed indicate that a parasitic causative agent is probably not involved in this melanoma.

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EXPLANATION OF THE PLATE.

(Photomicrography by S. C. Dunton).

PLATE I.

Fig. 1. Sagittal section through the caudal peduncle and tail of the silverside showing melanotic nodules (1, 2, 3,). H-E. About 25 ×.

Fig. 2. Higher magnification of a sagittal section through one of the melanotic nodules showing the extent of the lesion. **A**, point where melanin-bearing tumor cells had broken through the epidermis; **B**, thickened corium; **C**, slightly thickened epidermis with massed macrophages containing melanin granules; **D**, the fascia separating corium from muscle is broken and the pigmented cells invade the deeper layers of tissues; **E**, muscle fibers are split and the muscle tissue loses its striation and becomes hyalinized; **F**, bone showing the melanophores massed around the periosteal area; melanophores may also be seen in the region around the dorsal aorta; **G**, Scale. H.-E. 250 ×.